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Araştırma/ Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF PROPOLIS SAMPLES FROM THREE DIFFERENT REGIONS OF ANATOLIA

ANADOLU'NUN ÜÇ FARKLI BÖLGESİNDEN ALINAN PROPOLİS ÖRNEKLERİNİN ANTİMİKROBİYAL AKTİVİTELERİNİN İN VİTRO OLARAK İNCELENMESİ

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ABSTRACT

The aim of this study was to evaluate the antimicrobial activity of propolis samples from Zara/Sivas, Trabzon and Aydın regions in Turkey by the agar diffusion method. Antimicrobial activities of different propolis extracts (10 and 20%) were tested against *E. faecalis*, *S. aureus*, *E. coli* and *C. albicans*, in the present study. Microorganisms were inoculated into plates containing Mueller Hinton (MH) agar. Then round wells were punched in the cultivated agar plates and filled with test solutions. The MH agar plates were incubated for 24 h at 37°C and diameters of inhibition zones around each well were then measured and recorded. The test was repeated 7 times for each solution. The results of the present study showed that 2.5% sodium hypochlorite (NaOCl) and all propolis extracts showed antimicrobial activity against all microorganisms tested. However, 2.5% NaOCl was shown to be the most effective irrigant and statistically significant differences were found between 2.5% NaOCl and other experimental solutions against all microorganisms ($p<0.05$). When propolis extracts were evaluated, Zara propolis extracts showed better antimicrobial activity than other propolis extracts against all microorganisms tested. Also, there were statistically significant differences between 10 and 20% concentrations of propolis extracts from Zara and other propolis extracts against *E. faecalis* and *C. albicans* ($p<0.05$). Propolis extracts from Aydın showed better antimicrobial activity than Trabzon propolis extracts against *C. albicans* and *E. faecalis*. Also, significant differences were found between 10% concentration of propolis extract from Trabzon and 20% concentration of propolis extract from Aydın against *S. aureus* ($p<0.05$).

Key Words: Antimicrobial activity, NaOCl, propolis

ÖZET

Bu çalışmanın amacı, Türkiye'nin Zara/Sivas, Trabzon ve Aydın bölgelerinden alınan propolis örneklerinin, agar difüzyon yöntemi ile antimikrobiyal aktivitelerinin incelenmesidir. Çalışmada, farklı propolis ekstratlarının (%10 ve %20) *E. faecalis*, *S. aureus*, *E. coli* ve *C. albicans* üzerine olan antimikrobiyal aktiviteleri test edildi. Mikroorganizmalar Mueller Hinton (MH) agar içeren plaklara ekildi. Daha sonra ekim yapılan agar plaklarına kuyucuklar açıldı ve test solüsyonları ile doldurudu. MH agar plakları 37°C'de 24 saat inkübe edildi ve inhibisyon alanlarının genişlikleri ölçülerek kaydedildi. Her solüsyon için bu test 7 kere tekrarlandı. Çalışmanın sonucunda, %2,5'lik sodyum hipoklorit (NaOCl) ve tüm propolis ekstratlarının test edilen mikroorganizmalara karşı antimikrobiyal aktivitesinin olduğu görüldü. Bununla birlikte, %2,5'lik NaOCl'nin en etkili irrigan olduğu ve tüm mikroorganizmalara karşı diğer solüsyonlarla arasında istatistiksel olarak fark olduğu görüldü ($p<0,05$). Propolis ekstratları değerlendirildiğinde, Zara propolis ekstratlarının diğer propolis ekstratlarına göre test edilen bakterilere daha iyi antimikrobiyal etki gösterdiği bulundu. *E. faecalis* ve *C. albicans*'a karşı %10 ve %20 konsantrasyonlardaki Zara propolis ekstratları ile diğer propolis ekstratları arasında istatistiksel olarak fark vardı ($p<0,05$). Aydın propolis ekstratları, *E. faecalis* ve *C.albicans* üzerine Trabzon propolis ekstratlarına göre daha iyi antimikrobiyal etki gösterdi. Ayrıca, %10'luk Trabzon ve %20'lik Aydın propolis ekstratları arasında *S.aureus*'a karşı önemli fark bulundu ($p<0,05$).

Anahtar Kelimeler: Antimikrobiyal aktivite, NaOCl, propolis

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INTRODUCTION

The removal of remaining pulp tissue and dentinal debris and elimination of feasible microorganisms from the root canal system are of dominant magnitude during endodontic therapy. Root canals of symptomatic teeth with necrotic pulps and periapical bone destruction tend to harbor a larger number of bacteria and more complex anaerobic bacterial flora than the asymptomatic teeth with apical perodontitis.[1] Positive correlations have been found between the number of bacteria and clinical symptoms.[2,3] Failure to effectively eliminate them and their by-products might result in persistent irritation and impaired healing.[4] It has been widely reported that viable bacteria can remain within the canal system even after chemomechanical preparation.[5]

A variety of irrigant solutions have been used in endodontics in an attempt to eliminate or reduce the number of these bacteria. An endodontic irrigant should ideally exhibit powerful antimicrobial activity, dissolve organic tissue remnants, disinfect the root canal space, flush out debris from the instrumented root canals, provide lubrication, and have no cytotoxic effects on the periradicular tissues, among other properties.[6]

NaOCl is currently the most commonly used irrigant in endodontics, and its antimicrobial and tissue-dissolving property have been widely reported.[7,8] In addition, it is inexpensive, has a long shelf life, and is readily available. The inconvenience of using full-strength NaOCl as an endodontic irrigant has been correlated to its cytotoxicity if introduced beyond periradicular tissues. Its extrusion can cause excruciating pain, immediate swelling, and profuse bleeding.[9,10] Moreover, NaOCl has also been reported to inhibit bonding of resin-based sealers used as root canal filling material.[11] Therefore, an equally effective but safer irrigant is desirable.

Propolis, known as bee glue and bee propolis, is a brownish resinous substance collected by bees, mainly from plants. It is used to reinforce the combs and to keep the hive environment aseptic.[12] It is a potent antimicrobial, antioxidant, and anti-inflammatory agent. The main chemical elements present in propolis are flavonoids, phenolics, and various aromatic compounds. Flavonoids are wellknown plant compounds that have antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties.[13]

The purpose of this study was to evaluate the antimicrobial activity of 10 and 20% concentrations of propolis extracts from various regions (Zara/Sivas, Trabzon and Aydın) in Turkey, against selected microorganisms by agar diffusion method.

MATERIAL AND METHODS

Propolis samples

Propolis samples were collected from three different localities in Turkey, Zara/Sivas (Middle Anatolia), Trabzon (Northern Anatolia) and Aydın (Western Anatolia). Hand collected propolis were kept desiccated and in the dark up to their processing. Propolis samples were ground with an ultracentrifugal mill (Retsch, Haan, Germany), and 25 g powder was dissolved in 50 mL dimethyl sulfoxide (DMSO) (100%, w/v) by magnetic mixer for 24 h at 37°C. Working solutions at concentrations of 10% and 20% were then prepared in steril saline solution. Also, 2.5% NaOCl was used as the positive control and NaCl was used as negative control.

Test microorganisms

The following microorganisms were used to evaluate the antimicrobial activity, in this study: *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *C. albicans* (ATCC 10231). All microorganisms were provided by Cumhuriyet University, Faculty of Medicine, Department of Microbiology.

Antimicrobial activity test

Microorganisms were subcultured on broth brain heart infusion (BHI) agar and stored for 24 h at 37°C. Several colonies from the plates were taken to BHI broth which was incubated for 24 h at 37°C. Their density was adjusted to Mcfarland 0.5 ($1.5x10^8$ CFU mL^{-1}) by adding NaCl. 50 µL of each test microorganism suspension was then inoculated into plates containing MH agar and sterile loop was used to spread suspension across the surface of the agar media. Round wells, 5 mm deep and 6 mm in diameter, were punched in the cultivated agar plates and filled with 40µL of the test solutions. The tests were repeated 7 times for each solution tested. The

agar plates were incubated for 24 h at 37°C. Then, the diameters of microbial inhibition zones around each well were measured and recorded. Statistical analysis was carried out with analyses of variance (ANOVA) with Bonferroni test of multiple comparisons. The level of significance was set at p=0.05.

RESULTS

The agar diffusion method was used to determine the inhibition zones of the experimental solutions. According to the results in the Table 1; 2.5% NaOCl and all propolis extracts showed antimicrobial activity against all microorganisms tested. In contrast, NaCl was always ineffective.

When Trabzon and Aydın propolis extracts were compared, Aydın propolis extracts showed better results than Trabzon propolis extracts against *C. albicans* and *E. faecalis,* and there were statistically significant differences ($p<0.05$). While no significant differences were found between Trabzon and Aydın propolis extracts against *E. coli* ($p>0.05$), significant differences were found between 10% concentration of propolis extract from Trabzon and 20% concentration of propolis extract from Aydın against *S. aureus* ($p<0.05$).

Table 1. Mean area of the zones of microbial inhibition in mm (n=7) provided by experimental solutions

Microorganism	Z1	Z2	T1	T2	A1	A2	NaOCl	NaCl
E.faecalis	14.8*	13.3*	10.6 (Z1,Z2,NaOCl,NaCl,SS)	10 (Z1,Z2,A1,A2,NaOCl,NaCl,S)	11.7 (Z1,Z2,T2,NaOCl,NaCl,S)	11.4 (Z1,Z2,T2, NaOCl,NaCl,SS)	22.6*	0*
S. aureus	18.3 (T1,T2,A1,A2,NaOCl,NaCl,S)	17.3 (T1,T2,A2,NaOCl,NaCl,SS)	15.4 (Z1,Z2,NaOCl,NaCl,SS)	14.3 (Z1,Z2,A1,NaOCl,NaCl,SS)	15.9 (Z1,T2,NaOCl,NaCl,SS)	14.7 (Z1,Z2,NaOCl,NaCl,SS)	31.1*	0*
E. coli	16.1 (T2,A2,NaOCl,NaCl,SS)	13.6 (NaOCl,NaCl,SS)	14.7 (NaOCl,NaCl,SS)	12.7 (Z1,NaOCl,NaCl,SS)	13.7 (NaOCl,NaCl,SS)	13.3 (Z1,NaOCl,NaCl,SS)	38.5*	0*
C. albicans	20 (T1,T2,A1,A2,NaOCl,NaCl,S)	18.4 (T1,T2,A1,A2,NaOCl,NaCl,SS)	12.6 (Z1,Z2,T2,A1,NaOCl,NaCl,S)	9.6*	15.6*	13.6 (Z1,Z2,T2,A1,NaOCl,NaCl,S)	53.6*	0*

DISCUSSION

In the present study, the best antimicrobial activity was achieved by 2.5% NaOCl and statistically significant differences were found between 2.5% NaOCl and other experimental solutions against all microorganisms ($p<0.05$).

Among propolis solutions Zara propolis extracts showed best results against all microorganisms. There were statistically significant differences between 10 and 20% concentrations of Zara propolis extracts and other propolis extracts against *E. faecalis* and *C. albicans* ($p<0.05$). Also, significant differences were found between 20% concentration of Zara propolis extract and other propolis extracts against *S. aureus* except 10% concentration of Zara propolis extract ($p<0.05$). There were statistically significant differences between 20% concentration of Zara propolis extract and 10% concentrations of Aydın and Trabzon propolis extracts against *E.coli* ($p<0.05$).

In the present study, antimicrobial activities of two different concentrations (10% and 20%) of propolis samples from three different regions of Turkey were evaluated. The agar diffusion method was used to determine the inhibition zones of the different propolis extracts. This method has frequently been used to test the efficacy of various antimicrobials, because of it allows direct comparisons of root canal irrigants against the test microorganisms, indicating which irrigant has the potential to eliminate microorganisms in the local microenvironment of the root canal system. However, the outcome measure (ie, the zone of inhibition) is dependent on the ability of the test antimicrobials to diffuse in the agar.[14]

Among the procedures involved in the control of endodontic infection, irrigation is an important agent in eliminating microorganisms from the root canal system. Intracanal cleaning and disinfecting procedures are highly dependent on the mechanical

and chemical effects of the irrigants. Irrigant solutions in different concentrations with antimicrobial activity have been used during biomechanical instrumentation, particularly NaOCl. To date, NaOCl is the most commonly employed root canal irrigant and this irrigant has a broad spectrum of antimicrobial activity.[15,16] Similar to these previous studies, we found that 2.5% NaOCl showed the best antimicrobial effect on the microorganisms tested and statistically significant differences were found between 2.5% NaOCl and propolis extracts. However, alternative irrigation solutions have been used in endodontic treatment, because of toxic effects of NaOCl.[17,18]

Recently, propolis, product of bees wax, has been shown to possess antimicrobial and anti-inflammatory properties.[19] In dentistry, the use of propolis has been proposed in different areas including cariology,[20] oral surgery,[21] endodontics[19] and periodontology.[22] However, mechanisms of activity of propolis against microorganisms are still controversial. Some components present in propolis extracts like flavonoids (quercetin, galangin, pinocembrin, sakuranetin, kaempfreol) and caffeic acid, benzoic acid, cinnamic acid, probably act on the microbial membrane or cell wall site, causing functional and structural damages.[23-25] According to Amoros et al.[26] and Bonhevi et al.[27] its activity against microorganisms is more related to the synergistic effect of flavonoids (and other phenolics) than to the individual compounds.

Its antimicrobial efficacy also has been shown in the previous studies. Koo et al.[28] reported that, propolis extract showed high antimicrobial activity against *S. aureus* and *E. faecalis*; however, it showed only a slight inhibitory zone against *C. albicans*. Özan et al.[29] showed reported that 10% propolis extract was effective against *S. aureus*, *E. coli* and *C. albicans* after 20 minutes. Kujumgiev et al.[30] investigated the antibacterial (*S. aureus* and *E. coli*), antifungal (*C. albicans*) and antiviral activity of propolis samples from different locations. They found that all propolis extracts exhibited significant antibacterial (against *S. aureus*) and antifungal activity; however, no sample was active against *E. coli*. They stated that compounds of propolis extracts such as aromatic acids, phenolic acid esters, flavonoids, triterpenes, diterpenic acids and lignanes could be responsible for the beneficial properties of propolis. In another earlier study,[31] 10% propolis was found effective against all tested micrrorganisms (*P. nigrescens, F. nucleatum, A. israelii, C. perfringens* and *E. faecalis),* and *E. faecalis* was the least susceptible strain. Oncag et al.[32] observed that propolis had good in vitro antibacterial activity against *E. faecalis* in the root canals of extracted teeth, suggesting that it could be used as an alternative intracanal medicament.

In the present study, all propolis extracts were showed antimicrobial effect against all tested microorganisms. Agar diffusion test clearly indicated that propolis extracts from Zara had much more powerful antimicrobial activity compared with Trabzon and Aydın propolis extracts. Although, Trabzon and Aydın propolis extracts showed similar antimicrobial effects against *E. coli*, propolis extracts from Aydın were more effective than Trabzon propolis extracts against *C. albicans* and *E. faecalis*. *E. faecalis* was also more resistant microorganism against all irrigation solutions tested. These different antimicrobial effects of propolis samples might be connected to the constituents of propolis (flavonoids, aromatics, phenolics) that vary widely due to climate, season, location and year, and its nonstable chemical formula.[12]

Based on our results, we suggest that all propolis samples at 10 and 20% concentrations showed antimicrobial effect on selected microorganisms. However, 2.5% NaOCl was superior in its antimicrobial abilities when compared with propolis samples. Further laboratory and clinical investigations should be carried out to validate findings of beneficial use of propolis as intracanal medicament or as any other endodontic material.

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